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THE KARYOTYPE OF THE ABERRANT OBLIGATE ANT-ATTENDED MEALYBUG, *NEOCHAVESIA CALDASIAE* (COCCOIDEA: PSEUDOCOCCIDAE: RHIZOECINAE)

El cariotipo de *Neochavesia caldasiae* (Balachowsky) (Coccoidea: Pseudococcidae: Rhizoecinae), cochinilla aberrante exclusivamente atendida por hormigas

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ABSTRACT
We report information about the structure of the karyotype of the ant-attended mealybug *Neochavesia caldasiae* that maintains an obligate association with ants of the genus *Acropyga*. Seven pairs of holocentric chromosomes (2 = 14) constitute it, with one pair larger that the other six. This number is discussed according the to information available about the Pseudococcidae karyotype evolution, focusing the Rhizoecinae.

Key words. Chromosome number, mealybug, evolution, Neotropical mutualism.

RESUMEN
Se registra información sobre la estructura del cariotiopo de la cochinilla *Neochavesia caldasiae* atendida por hormigas del género *Acropyga*. Se trata de siete pares de cromosomas holocéntricos con un par más largo que los demas. Este número se discute de acuerdo a la información disponible sobre la evolución cariotípica en Pseudococcidae con énfasis en Rhizoecinae.

Palabras clave. Número cromosómico, cochinilla, evolución, mutualismo neotropical.

INTRODUCTION

The ant-attended mealybug *Neochavesia caldasiae* (Balachowsky 1957) (Coccoidea: Pseudococcidae: Rhizoecinae) has been reported from Colombia, from where it has been described, Trinidad and Brazil (Balachowsky 1957, Williams & Granara de Willink 1992, Johnson *et al.* 2001, Delabie, unpublished results). This insect has an obligate association with Neotropical ants *Acropyga* (Formicidae; Formicinae; Plagiolepidini) (Williams 1998, Johnson *et al.* 2001). This symbiosis, product of a coevolution process which occurs at least since the Miocene, is so well established that the ant females systematically carry a fertilized female of the mealybug during the nuptial flight (Flanders 1957, Eberhard 1978, Campos & Morais 1986, Johnson *et al.* 2001). The three known species of *Neochavesia* present an aberrant scorpion-like body form (Williams 1998), that seems to be adapted to ant manipulation and transport. The same is observed in the two other genera of the Rhizoecinae which to live symbiotically with the ants *Acropyga*, *Eumyrmococcus* and *Xenococcus* (Williams 1998). A strong argument which confirms the great originality of this group (and its primitive character too) is that the only cases of true female pupal stage occurrences in all Coccoidea are known exclusively in these same Rhizoecinae genera: *Eumyrmococcus*, *Xenococcus* (Williams 1998) and *Neochavesia* (J.H.C. Delabie, unpublished results). In *N. caldasiae*, the reproduction has been confirmed to be sexual and both sexes co-occur in the ant nest chambers (J.H.C. Delabie, unpublished results).

Ants and mealybugs live together in subterranean nests along the roots of tropical and subtropical plants from which the coccids extract the sap, basis of their diet. The ants take of the honeydew at pseudococcids produce and probably of the insect itself for food (Delabie *et al.* 1991, Delabie 2001). *N. caldasiae* is known to live in association with the ant *Acropyga robae* Donisthorpe on the roots of coffee and cocoa trees in Colombia and Trinidad (Williams 1998), and with *Acropyga berwicki* Wheeler on the roots of cocoa trees and other plants in southern Bahia, Brazil (Delabie 2001).

Karyological studies in Pseudococcidae are scarce (Hughes-Schrader 1948, Nur 1980, Nur *et al.* 1987). Chromosome number and other information about the mealybug cytogenetics are available today for 90 species (on an amount of around 2,000 known species for the whole family) (Nur *et al.* 1987). We report the karyotype of *N. caldasiae*, in order to contribute to the comprehension of the very uncommon biology of this mealybyg.

MATERIAL AND METHODS

The ant nests containing with the two groups of organisms (ants and mealybugs) were collected in the experimental fields of the Cocoa Research Center (CEPEC) of CEPLAC Institution (14^{o}45'S, 39^{o}13'W), at Ilhéus, Bahia State, Brazil, in March 2001. The nests have been carried alive to the Laboratory of Cytogenetics at the Federal University of Viçosa, MG, Brazil, where the mealybugs were separated for study.

Dr. Danièle Matile-Ferrero (Museum National d'Histoire Naturelle, Paris, France) and Dr. Penny J. Gullan (University of California, Davis, USA) confirmed both the mealybug identification.

Chromosome number and their characteristics were observed in young female embryonic cells (20 individuals) prepared by tissue dissociation, according to Nur *et al.* (1987), from newly laid eggs. Presumably, the concerned tissues were epidermis, digestive and nervous systems because these organs show high mitotic activity in this developmental stage on histological analysis of the embryo. Occasional observations were made too on mycetome cells and on few male-producing eggs. The preparations were observed under light microscope and pictures have been taken with a digital camera coupled to an image analyzer software Image-Pro-Plus 4.0 (Cybernetics Ltd. 1992).

RESULTS AND DISCUSSION

Chromosomes of *N. caldasiae* are holocentric as in the other Pseudococcidae, as well as in all hemipterans (White 1973, Nur 1980), giving them the appearance of short batons. We found seven pairs of chromosomes (2n = 14) (figure 1) in the all metaphases analyzed in the female individuals. No special feature allowed distinguishing the chromosome pairs among them, although a discrete gradual length variation is observed. The constrictions that appear in few of them on the figure are due to the microscopic preparation and condensation of these very little chromosomes.

Observations on numerous cells of a male tissue, apparently in synchronous differentiation, showed n = 7, that are certainly metaphases of the second meiotic division. The mycetome cells of *N. caldasiae* showed to possess a larger number of chromosomes, possibly tetraploid. This tissue, made of very large cells in the imagoes, contains endosymbionts as in many hemipterans (Tremblay 1977), which migrate from the mother to the egg at an early stage of its formation, as observed on histological preparations of fertilized females (J.H.C. Delabie, unpublished results).

Chromosome number (2n = 14) is in the range of the data available for the Pseudococcidae, that is, from 8 to 64 with modal number = 10 being chromosome number >20 occasional (Nur 1980, Nur *et al.* 1987). The modal number of chromosome in different families of Hemiptera is possibly the ancestral condition (Nur *et al.* 1987). For the mealybugs, 2n = 10 may be the putative number, because 68% of the studied taxa present this character and karyotypes with 2n = 10 exist in all the major taxonomic levels of the family (Nur *et al.* 1987). Between the Pseudococcidae and according the same authors, 2n = 14 have been reported only in four species: *Clavicoccus tribulus* Ferris, *Nairobia bifrons* De Lotto, *Nesopedronia cibotii* (Beardsley) and *Paraputo anomala* (Newstead). This chromosome number has been also reported in *Puto* sp. (Hughes-Schrader 1948), sometimes placed in a separated primitive family (Putoidae) or maintained in the Pseudococcidae (Ben-Dov 1994).

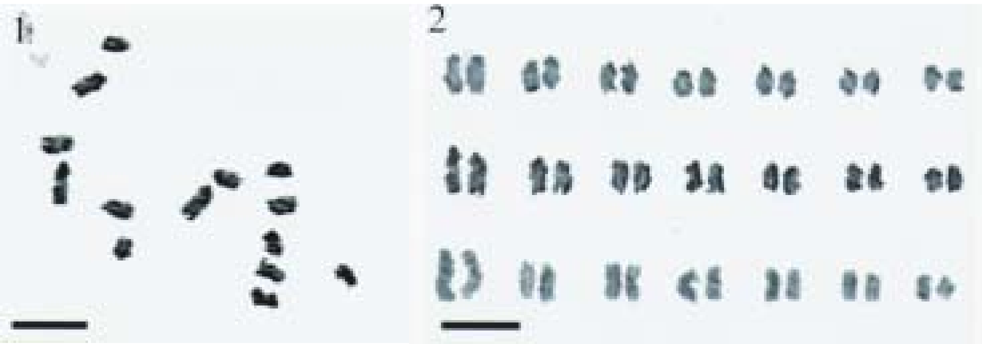

Figure 1. Citogenetic study of *Neochavesia caldasiae*. 1: metaphase, 2n=14; 2: karyogrames of 3 females with 2n=14, Coloration: Giemsa, bar = 5 µm.

Rhizoecinae aggregates 10 genera of exclusively subterranean ("cryptic" in the ecological sense) Pseudococcidae living on the roots of plants, with many species being reported to be associated with *Acropyga* ants (Williams 1998). In the subfamily, only three species of the non-obligate ant-attended *Rhizoecus* (Williams 1998): *Rhizoecus falcifer* Knuckel d'Herculais, *Rhizoecus mayanus* (Hambleton) and *Rhizoecus dianthi* Green, have cytogenetical data, with respectively, 2n = 12, 10 and 12 (Nur *et al.* 1987). As pointed out by the same authors, in mealybugs, increase in chromosome number from the ancestral condition (2n = 10) occurs more often that decreases and the most probable model of the evolution of their karyoptypes should be both by fission and/or polyploidization. The 2n = 14 found in *N. caldasiae*, may results of a double fission from the ancestral condition.

An important conclusion of Nur *et al.* (1987) was that, in mealybugs, any species with a chromosome number different from the modal number (2n = 10) has great probability to be a monotypic genus. This is obviously not the case for *Neochavesia* with 3 known species, according Williams (1998). Other conclusions of the same authors were that such a species should be less likely to become extinct, should be well fixed phylogenetically with few chance to evolve in new taxa, and the morphological changes associated to the generic evolution should be dependant from the haploid number. Due to the extreme specialization and dependence of ants of *Neochavesia*, product of a coevolution with *Acropyga* of at least 20 millions years (Johnson *et al.* 2001), these last arguments should be certainly applicable to this genus.

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